

A Survey for Nymphs of Host-Seeking *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) on Tree Trunks in Deciduous Forests

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ABSTRACT Trunks of 83 trees in a mixed deciduous forests in Maryland were sampled for the presence of nymphs of the blacklegged tick, *Ixodes scapularis* Say, and the lone star tick, *Amblyomma americanum* (L.). Although one or more nymphs of either *I. scapularis* or *A. americanum* was found in leaf litter and substrate ≤ 1 m from the bases of 47% of the trees sampled, a total of 6 *I. scapularis* nymphs was found on the trunks of only five trees. No nymphs were found on the trunks of 12 dead trees. No *A. americanum* nymphs were found on any tree trunks. The trunks were sampled to 2.5 m above the soil, but the nymphs were found ≤ 1 m from the ground. More than 50% of *I. scapularis* nymphs found in the leaf litter ≤ 1 m from bases of living trees were north of the trees sampled, whereas few *I. scapularis* were found west of trees. These findings suggest that the *I. scapularis* nymphs' presence on tree trunks is of little ecological consequence, unless nymphs were being removed from tree trunks by acquiring hosts at such a rapid rate that nymphal numbers on trunks could not accrue.

KEY WORDS *Ixodes scapularis*, blacklegged tick, *Amblyomma americanum*, lone star tick, host-seeking, Lyme disease

MOST CASES OF Lyme disease result from persons being bitten by nymphs of the blacklegged tick, *Ixodes scapularis* Say, that are infected with the spirochete, *Borrelia burgdorferi* Johnson, Schmidt, Hyde, Steigerwalt & Brenner (Burgdorfer et al. 1982). Lone star ticks, *Amblyomma americanum* (L.), coexist with *I. scapularis* at many locations on the coastal plain of mid-Atlantic states. Lone star ticks are vectors of the *Ehrlichia* species that cause human monocytic ehrlichiosis and *I. scapularis* is considered to be a vector of the *Ehrlichia* species that causes human granulocytic ehrlichiosis (Dumler and Bakken 1995). Larvae and nymphs of *I. scapularis* feed on a wide variety of vertebrate hosts (Main et al. 1982). Prominent among these hosts are species that often forage and nest arboreally (white-footed mice, *Peromyscus leucopus*; gray squirrel, *Sciurus carolinensis*; raccoon, *Procyon lotor*). White-footed mice are considered a major host and reservoir for *B. burgdorferi* (Spielman et al. 1985).

Host-seeking nymphs and larvae of *I. scapularis* are generally associated with leaf litter, whereas adults may wait for hosts as high as 1 m above ground. Larval and nymphal ticks that ascend slender vegetation (e.g., grass stems) must contend with the problem of maintaining their water balance when exposed to drying breezes (Fleetwood and Teel 1983). When the trunks of trees (≥ 3.18 cm

diameter at breast height [dbh]) that had ≥ 5 larvae in the leaf litter within 1 m of their bases, were sampled for larvae, 45.8% of the trees had ≥ 1 larva on the trunks (Carroll 1996). Larvae probably get on the trunks by climbing from the leaf litter, where tick eggs are deposited, or by falling or being groomed off a host that was on the tree. Blacklegged tick nymphs can get on tree trunks by these ways, but they might also arrive there by dropping off as engorged larvae in the arboreal nests of hosts. Host-seeking *I. scapularis* nymphs are known to be associated with stone walls and fallen logs where larval hosts might nest (Matuschka et al. 1991, Stafford and Magnarelli 1993, Carroll and Kramer 2001). The purpose of this study was to learn whether host-seeking nymphs of *I. scapularis* and *A. americanum* occurred on tree trunks as had been reported previously for larvae of *I. scapularis*.

Materials and Methods

The study was conducted in 2000 in upland mixed hardwood forest at the U. S. Fish and Wildlife Service, Patuxent Wildlife Research Refuge, Laurel, MD, during the summer host-seeking season for *I. scapularis* and *A. americanum*. A dense population of white-tailed deer, *Odocoileus virginianus*, principal host of the adult stage of *I. scapularis* and *A. americanum*, supported an established population of the former

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Table 1. Occurrence of host-seeking nymphs on tree trunks and in leaf litter 1 m from bases of tree trunks

Species	n	Mean diam, cm	No. trees with ≥1 <i>I. scapularis</i> nymph on trunk	No. trees with ≥1 nymph in leaf litter ≤1 m from base	
				<i>I. scapularis</i> nymph	<i>A. americanum</i> nymph
Tupelo, <i>Nyssa sylvatica</i>	20	13.9 ± 1.9	1	8	1
Black oak, <i>Quercus velutina</i>	14	41.6 ± 4.9	2	5	1
White oak, <i>Q. alba</i>	10	35.1 ± 4.1	1	2	1
Red maple, <i>Acer rubrum</i>	10	24.0 ± 4.6	1	1	3
American beech, <i>Fagus grandifolia</i>	7	37.6 ± 7.5	0	5	1
American holly, <i>Ilex opaca</i>	5	17.9 ± 2.8	0	4	0
Sassafras, <i>Sassafras albidum</i>	2	8.1 ± 0.3	0	0	0
Sweet gum, <i>Liquidambar styraciflua</i>	2	16.4 ± 6.7	0	1	0
Loblolly pine, <i>Pinus taeda</i>	1	29.9	0	1	0
Standing dead trees (species uncertain)	12	30.4 ± 2.5	0	7	1

There were no significant differences in nymph counts among tree species after adjusting for area searched.

species and a burgeoning population of the latter in the study area. The trunks of 71 living and 12 dead trees, having circumferences at breast height ≥15 cm (≥4.9 cm dbh), were sampled for nymphs by wrapping a white flannel cloth (0.5 by 0.5 m) around the trunk and pressing the cloth into crevices in the bark. After the cloth was examined for ticks, it was repositioned laterally, if needed, to sample the entire circumference at that the same height and then upward with as little overlap of areas already contacted as possible. Nymphs were readily discernible on the cloth, so there was little likelihood of picking up a nymph at one height and not noticing it until after sampling another height. Ticks were removed from the cloth and placed in vials for identification. Vertical distribution of nymphs on the tree trunks was determined by sampling in five zones: ≈0.08–0.58, 0.58–1.08, 1.08–1.58, 1.58–2.08, and 2.08–2.58 m above ground level. Because the bases of many trees, especially the larger ones, were covered with mosses that spread upward from the ground, the basal ≈8 cm of trunks were not sampled. The inner surfaces of tree holes were not sampled. The circumference at breast height of each tree was recorded, as was its species and whether or not a tree hole (at any height) was detected. The compass direction in relation to the center of a tree trunk on which a tick was found was recorded. Sampling was done in the afternoons (1300–1700 hours) when vegetation was dry and temperatures were 28–32°C.

Trees were sampled in five transects each 30–50 m long and 0.2–1.3 km apart in which successive nearest neighboring trees with trunks of diameter >5 cm were examined. Each transect was in an area that had been presampled for the presence of nymphs by flagging the leaf litter and substrate with a tick sweep (flannel cloth, 0.5 by 0.5 m). Transects were not established in areas where nymphs were not found by flagging. Immediately before sampling each tree trunk for ticks the leaf litter and substrate within ≈1 m of the base of the trunk was flagged for nymphs. The flagging around the base of each tree was done in four roughly equivalent zones (quadrants) related to each quadrant's compass direction with the tree trunk as the center point. The quadrants were sampled in random order

for each tree. The distribution of *I. scapularis* nymphs, by the four compass directions, was analyzed using the GENMOD procedure (log link function) (SAS Institute 1999). A Poisson distribution for the count of the nymphs was assumed, because the dispersion parameter was estimated to be near 1. Two additional independent variables that might predict the count of nymphs are species of tree and the total area searched for each species/direction combination (for an individual tree this was within 1 m of the trunk). These variables were also tested for significance using the GENMOD procedure. The number of *I. scapularis* nymphs on trunk surface (0.08–1.08 m above ground level) was compared with the number of nymphs on the ground (leaf litter) within 1 m of the bases of the trunks, with the log of the area searched as a covariate, using the GENMOD procedure.

Results

Only six *I. scapularis* and no *A. americanum* nymphs were found on tree trunks. The *I. scapularis* nymphs occurred on five trees representing four species of trees: black oak, *Quercus velutina*; white oaks, *Q. alba*; red maple, *Acer rubrum*; black gum (tupelo), *Nyssa sylvatica* (Table 1). Four of the nymphs were in lowest sample zone (0.08–0.58 m above ground level) and the other two nymphs were found 0.58–1.08 m above ground (Table 2). The trees on which nymphs were found were 36.8–69.6 cm dbh (Table 3). No nymphs were found on any dead trees (*n* = 12), and only one nymph was found on the seven living trees that had ≥1 hole in their trunks large enough to be the entrance-way for a nest of a host. Three of the six nymphs found on tree trunks were captured on the north sides of the trunks.

Table 2. Vertical distribution of host-seeking *I. scapularis* nymphs on tree trunks

	Meters above ground				
	0.08–0.58	0.58–1.08	1.08–1.58	1.58–2.08	2.08–2.58
No. nymphs	4	2	0	0	0
No. trees with ≥1 nymphs	3	2	0	0	0

Table 3. Numbers of *I. scapularis* nymphs found on different size classes (diameters) of tree trunks

	Diameter of trees							
	<10	10–20	20–30	30–40	40–50	50–60	60–70	70–80
No. trees samples	17	16	14	11	4	5	3	1
No. nymphs found	0	0	0	1	3	1	1	0

Flagging leaf litter (including above ground tree roots) within 1 m of the bases of the trees whose trunks were sampled, captured 43 *I. scapularis* nymphs with 32 (45.1%) of the living trees having ≥ 1 nymphs of either species near its base. Ten of the nymphs captured from the leaf litter near sampled trees were *A. americanum*. There were ≥ 1 nymphs near the bases of all seven American beeches, *Fagus grandifolia*, sampled and around four of five hollies, *Ilex opaca*, but near just two of 10 white oaks, *Q. alba*. Among the dead trees, 58.3% had ≥ 1 nymph of either species around their bases. Just two (6.3%) of the 32 living trees having ≥ 1 *I. scapularis* nymphs near its base had ≥ 1 *I. scapularis* nymphs on their trunks, similar to the 7% observed for all living trees whether or not any nymphs were found near their bases. Six *I. scapularis* nymphs were found on a total trunk area of 58.6 m², resulting in a density of 0.102 nymphs/m², and 34 nymphs were found on a total ground area of 282.0 m², resulting in a density of 0.121 nymphs/m². Assuming similar efficiencies of the flannel cloth in capturing ticks from bark and leaf litter, the numbers of *I. scapularis* nymphs on tree trunks (0.8–1.08 m above ground) and on the ground (within 1 m of base of trunk) did not differ significantly (GENMOD procedure, including log of area searched as a covariate, $P > 0.26$). *Ixodes scapularis* nymphs were found in significantly greater numbers ($P < 0.05$) north of the tree trunks than in the other compass directions. Eighteen (52.9%) nymphs of *I. scapularis* in the leaf litter ≤ 1 m from the bases of living trees were in the northern quadrant (trunk was center point) relative to the base of the tree, compared with seven (20.6%) nymphs in the east, six (17.6%) south and three (8.8%) west. For trees with diameters > 20 cm dbh, 63.2% (12 of 19) of *I. scapularis* nymphs found in the leaf litter were northward and just one nymph (5.3%) was found westward. Nymphs of *I. scapularis* found around the bases of dead trees were rather evenly distributed among compass directions (one to three nymphs per direction). As expected, the size of the area searched was a significant covariate in the model. Species of tree, was not a significant predictor of nymph count, however, because the number of nymphs per tree was low, this test lacked power. Although counts for nymphs of *A. americanum* in the leaf litter around tree trunks were too low for a meaningful statistical analysis, the numbers of *A. americanum* nymphs captured were evenly distributed among the quadrants with two nymphs in the north, three east, three south, and two west.

Discussion

The occurrence of nymphs of *I. scapularis* and *A. americanum* on tree trunks appears to be an infrequent event with just 7% of living trees (6% of living plus dead trees) sampled having ≥ 1 nymph between 0.08 and

2.58 m above ground level. Even when ≥ 1 nymphs were found in leaf litter within one m from the bases of trees, few of these tree trunks (6.3%) had ≥ 1 nymphs on them. Only 9.9% of the living trees had at least one *A. americanum* nymph within 1 m of their bases. The low numbers of nymphs on tree trunks may simply reflect the chance that a wandering nymph encounters the base of a tree given the overall density of nymphs at the locations sampled. When the density of *I. scapularis* nymphs per square meter on tree trunks (0–1 m above ground) was compared with the density of nymphs around the bases of trees, no significant difference was found. This assumes that sampling tree trunks with flannel cloths was as effective in capturing nymphs as sampling leaf litter with flannel cloths attached to a pole. No nymphs were found above one m above ground level. The white-footed mouse, *P. leucopus*, a major host of *I. scapularis* larvae and nymphs frequently forages and nests in trees (Nicholson 1941). Fed larvae of *I. scapularis* are reported to tend drop off their rodent hosts while the hosts are at rest, which would be in their nesting sites (Matuschka et al. 1991). Numerous scratches made by the claws of squirrels were observed on the bark of the trunks of trees sampled, suggesting that nymphs on tree trunks can contact suitable hosts. Nymphs on tree trunks may not be as subject to adverse micrometeorological conditions (e.g., low relative humidity) as on an exposed blade of grass or twig (Milne 1950, Fleetwood and Teel 1983, Loye and Lane 1988, Harlan and Foster 1990, Ocabo-Melendez et al. 1995, Vail and Smith 1998, Randolph and Storey 1999). The bark of many species of trees is layered and fissured providing numerous humid refugia where ticks could rehydrate themselves (Yoder and Spielman 1992). Precipitation was in the normal range for early summer during this study, so the ticks were not subjected to unusually desiccating conditions during the course of the study. No nymphs were found on dead trees, even though the dead trees had many cavities that might serve as nests for hosts, and just one nymph was found on living trees that had ≥ 1 hole in its trunk or branches that might be an entrance to a nest. Because of the overall infrequent occurrence of nymphs on tree trunks (dead and alive), it could not be determined whether the presence of a tree hole influenced the likelihood of host-seeking nymphs occurring on the trunk. Flagging showed that slightly more than half (58.3%) of the dead trees had at least one nymph within one m of their bases which was similar (45.1%) to the living trees.

All the *I. scapularis* nymphs found on tree trunks were found 0.08–1.08 m above ground level. This contrasts with the case of *I. scapularis* larvae in which 15% of 101 larvae on tree trunks were found 1.08–2.08 m above ground level (Carroll 1996). Larvae of *I. scapularis* appear to be less capable of dispersing several meters than nymphs (Daniels and Fish 1990, Carroll

and Schmidtmann 1996), but seem to ascend tree trunks as high or higher than nymphs (Carroll 1996). The distribution of *I. scapularis* among trees according to trunk diameter resembled that of larvae in that ticks tended to be found on trees with larger trunks (Carroll 1996). All nymphs were found on the top 23% percentile of trees ranked by trunk diameter. Three of the six *I. scapularis* nymphs collected from tree trunks were from the north sides of the trees, but more interestingly 52.9% (18 of 34) of the blacklegged tick nymphs found in the leaf litter around the bases of living trees were from the northern quadrant. With trees having diameters > 20 cm, 63.2% of *I. scapularis* nymphs in the nearby leaf litter were found northward and just 5.2% westward. No such relationship seemed to exist for *I. scapularis* nymphs and dead trees and with *A. americanum* nymphs and living trees. Even though the forest canopies were rather continuous at the study sites, it appeared that *I. scapularis* nymphs might optimize their micrometeorological environment by avoiding exposure to even limited direct insolation and associated co-variants (e.g., temperature) (Harlan and Foster 1990). Around standing dead trees the continuity of the canopy tended to be broken, allowing greater insolation of the leaf litter.

The risk that a person might acquire a host-seeking *I. scapularis* nymph by leaning against a live tree or a standing dead tree appeared to be far less than from sitting on a fallen log (Carroll and Kramer 2001) or a stone wall (Stafford and Magnarelli 1993). Because nymph numbers per square meter were similar for the lower portion of tree trunks and the leaf litter, there exists some danger of acquiring a host-seeking nymph if a person contacts a tree trunk, particularly nearer the base of a tree. The extent to which most persons contact tree trunks is much less than the contact they make with leaf litter when walking and standing in woods. Concentrated host activity could result in extremely high host acquisition rates on tree trunks and confound this interpretation. The association between host-seeking nymphs and the trunks seems even less significant than that of larvae and tree trunks.

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